

ORIGINAL ARTICLE

Inter-relationships of *Salmonella* Status of Flock and Grow-Out Environment at Sequential Segments in Broiler Production and Processing

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Impacts

- *Salmonella* status of broiler carcasses in the processing plant is associated with recovery of *Salmonella* from grow-out house environmental and broiler samples collected pre-harvest.
- Likelihood of *Salmonella* recovery from broiler carcasses exiting the immersion chill tank is associated with *Salmonella* status of the house litter at the time of harvest and prior to placement of the birds.
- The immersion chilling of broiler carcasses disrupts some of the relationships between the processing plant and pre-harvest samples.

Keywords:

Salmonella; broiler; food safety; epidemiology; risk analysis

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Summary

In this study, we investigated how the likelihoods of *Salmonella* presence in various samples from broilers and their grow-out environment throughout one production cycle were related. Sixty-four broiler flocks from 10 complexes of two companies in the southern United States were included in the study. Samples from the gastrointestinal tracts of chicks, transport tray pads and litter and drag swabs from the house were collected on the day of placement of each flock. Approximately, 1 week before harvest, whole bird carcass rinses, caecum and crop samples were collected from birds from these same flocks. On the day of harvest, litter and drag swab samples were also taken from the house after the birds were removed. Upon arrival of the flocks at the processing plant, whole carcass rinses, caecum and crop samples were collected. As the flocks were processed, carcass rinses were collected just before the carcasses entered the immersion chill tank and as they exited the chill tank. Logistic regression was used to model the relationships between the likelihood of *Salmonella* in samples of each type collected at each sampling point and *Salmonella* frequencies in all the samples taken from the flock and grow-out environment at preceding production stages. The analysis demonstrated that increased likelihood of *Salmonella* contaminated carcasses entering the immersion chill tank was associated with higher contamination of the exteriors and crops of birds at arrival for processing as well as house environmental samples at the time of harvest and prior to placement. The best predictors of post-chill broiler carcass *Salmonella* status were the frequencies of *Salmonella* in the litter on the day of harvest and prior to placement. The immersion chilling appeared to disrupt some of the relationships between the processing plant and pre-harvest samples.

Introduction

Reducing the frequency of *Salmonella* in broilers is important from the standpoint of both consumer protection and industry sustainability. There is disagreement in the published literature as to which, if any, pre-harvest segment of the broiler grow-out production continuum is the most crucial in pre-disposing *Salmonella* contamination of the carcasses exiting the immersion chill tank. Several studies have demonstrated associations among *Salmonella* contamination of broiler breeders, in the hatchery, or of day-old birds with *Salmonella* status during grow-out and/or processing within integrated poultry operations (Morris et al., 1969; Bains and MacKenzie, 1974; Bhatia and McNabb, 1980; Higgins et al., 1981; Goren et al., 1988; Blankenship et al., 1993; Christensen et al., 1997; Chriel et al., 1999; Rose et al., 1999; Skov et al., 1999; Bailey et al., 2001, 2002; Cardinale et al., 2004). Other work has shown that factors important in forming the *Salmonella* profile of processed flocks were *Salmonella* contamination of the previous flock in the grow-out house, of the house before placement of the flock, of the flock during grow-out, or even *Salmonella* introduced at the time of transportation to the plant and in processing (Lahellec and Colin, 1985; Goren et al., 1988; Rose et al., 1999, 2003; Heyndrickx et al., 2002; Cardinale et al., 2004). Furthermore, one study concluded that the identity of the processing facility itself is a major risk factor associated with *Salmonella* contamination of the processed broiler carcasses (Heyndrickx et al., 2002). Several studies have suggested that cross-contamination with *Salmonella* can occur from within a flock as well as from flocks previously processed on the line (Morris et al., 1969; Dougherty, 1976; Sarlin et al., 1998). Many authors have emphasized the complexity of sources and conditions of exposure or contamination as they relate to the resulting *Salmonella* status of broiler flocks (Bhatia and McNabb, 1980; Lahellec and Colin, 1985; Davies and Wray, 1994, 1996, 1997; Jacobs-Reitsma et al., 1994; Byrd et al., 1999; Chriel et al., 1999; Rose et al., 1999, 2000, 2003; Skov et al., 1999; Nauta et al., 2000; Bailey et al., 2001; Davies et al., 2001; Heyndrickx et al., 2002; Cardinale et al., 2004; Liljebjelke et al., 2005).

The objectives of this study were to determine the occurrence of *Salmonella* in different sample types collected from broiler flocks and house environments at sequential segments of one grow-out production cycle and to investigate associations among the sample types and locations. Of particular interest was determining how the *Salmonella* status of the flock and environment at earlier segments of production were related to *Salmonella* contamination of broiler carcasses just prior to entering

the immersion chill tank and, especially, those exiting the chill tank at the end of processing.

Materials and Methods

Sampling strategy

Sample collection was carried out during 2003–2006 in the southern United States (US). Two commercial poultry companies with flocks in Mississippi, Alabama, Louisiana and Texas collaborated in the study. One company was made up of four and the other of six complexes. The two companies were selected for participation in the study based on the previous working relationships and on consideration that they were representative of the regional industry. In one company, four grow-out farms from each of three complexes and three farms from the fourth complex were selected for a total of 15 farms. In the other company, five farms from each of two complexes, four farms from each of three complexes and one farm from the sixth complex were selected for a total of 23 farms. The sampled farms were selected by the companies prior to the placement of the broiler flocks so that the flocks would be processed on a Monday or Tuesday to facilitate laboratory and transportation logistics. Two houses on each of the 38 farms were selected prior to placement of new flocks for a total of 76 houses.

The goal of the sampling strategy was to follow each of 76 flocks from the day of placement through harvest and processing. An overview of the sampling scheme is presented in Table 1. From each flock, environmental and/or bird samples were collected on the day of placement of the chicks in the house, approximately 1 week before harvest and on the day of harvest and processing. As will be discussed in more detail later, some flocks were lost from the study so that 64 of the original 76 flocks made it through all stages of sampling.

Broiler houses

The houses selected for sampling were usually a house on the end of a row and the adjacent house. The lengths of sampled houses ranged from 110 to 152 m, with the most common lengths being 128 and 152 m; half of the houses were 12 m and the other half 13.4 m wide respectively. The houses were constructed in such a way that the length of the houses was oriented east and west.

Sample collection

Day 1 litter samples and drag swabs

Four litter samples (D1/LR) were collected within 2 h prior to placement of the flock in the house. Each litter sample consisted of eight individual samples that were

Table 1. Sampling strategy

Sample day	Number of flocks	Number and type of samples collected per flock ^a						
		Drag swabs	Litter samples	GI Tract samples	Tray pads	Carcass rinses ^b	Caeca samples	Crop samples
Day of placement	76	4 D1/DS	4 D1/LR	30 D1/GI	30 D1/TP			
1 week before harvest	70					30 GO/WCR	30 GO/CA	30 GO/CP
Day of harvest								
Post-harvest	68	4 PH/DS	4 PH/LR					
Arrival at processing plant	66					30 PA/WCR	30 PA/CA	30 PA/CP
Pre-chill tank	66					30 PR/CR		
Post-chill tank	64					30 PO/CR		

^aThe number of samples of each type collected per flock is followed by the abbreviation used in the other tables to designate the point in the production continuum where the sample was taken and the sample type.

^bCarcass rinses conducted 1 week before harvest and upon arrival at the processing plant were performed with whole, feathered carcasses. Carcass rinses conducted pre- and post-chill tank were performed on eviscerated carcasses with feathers, head and feet removed.

collected equidistantly along one of four lines parallel to the long side of the house and then placed into a Whirl-Pak[®] Bag (NASCO, Fort Atkinson, WI, USA). Four drag swabs (D1/DS) were collected by dragging two swabs down and back along two lines parallel to the long side of the house on one side of the house and then repeating on the other side of the house. The drag swabs were prepared, collected and processed as previously described (Kingston, 1981; Opara et al., 1992; Caldwell et al., 1994; Rybolt et al., 2005). Briefly, each swab was made with 10.2 × 10.2 cm cotton gauze (Abco Dealers Inc., Nashville, TN, USA). A swab was tied to 182.9 cm of cotton-polyester string (The Lehigh Group, Macungie, PA, USA). The swab and string were steam-sterilized and aseptically transferred into a sterile Whirl-Pak[®] Bag containing 20 ml sterile double strength skim milk. The latter was prepared according to the instructions from the manufacturer (Wal-Mart Stores, Inc., Bentonville, AR, USA), but with double the concentration of milk powder to water, which was 91 g per 500 ml. Samples were transported to the laboratory on wet ice. Upon arrival at the laboratory, within 8 h of the sample collection, 25 g of each litter sample was placed into a Whirl-Pak[®] Filter Bag (NASCO, Fort Atkinson, WI, USA); 225 ml of buffer peptone water (BPW) was added, mixed for 1 min and incubated at 42°C overnight. One hundred millilitre of BPW was added to each drag swab sample, the bag was mixed and the sample was incubated at 42°C overnight.

Day 1 chick gastrointestinal tract samples and tray pads

Sampled flocks, numbering from 16 000 to 27 500 birds each, were transported from the hatchery to grow-out farms in plastic transport trays, containing an average of 100 chicks. The bottoms of the trays were covered with single-use paper pads for 74 of the 76 flocks. The chicks were transported to the farms in climate controlled semi-

trailers or buses. As each flock was placed in its house, 30 transport trays were set aside by the placement crew. One chick was selected at random from each of the 30 sampled trays and humanely killed by cervical dislocation. The carcass was placed into a sterile Whirl-Pak[®] Bag. The transport tray pads (D1/TP) from the 30 trays were individually aseptically collected. Each pad was torn in half and one half was placed into a sterile Whirl-Pak[®] Bag. Samples were transported to the laboratory on wet ice within 6 h of the sample collection. Upon arrival at the laboratory, the gastrointestinal tract from each chick carcass (D1/GI) was aseptically removed and placed into a sterile Whirl-Pak[®] Filter Bag with 22 ml of buffered peptone water (BPW). After the sample was stomached for 60 s, 10 ml was aseptically removed for other purposes, and the rest was incubated at 42°C. One hundred millilitre of BPW was added to each transport tray pad sample, stomached for 60 s and incubated at 42°C overnight.

End of grow-out whole carcass rinse, caeca and crop samples

Four flocks raised at two farms were lost from the study due to Hurricane Katrina in the fall of 2005. Two other flocks raised at a single farm were lost due to industry-related issues. Seventy of the original flocks from 35 farms were sampled at the end of the grow-out, approximately 1 week before harvest. At this time, the broiler flocks were from 41 to 57 days old, with an average of 49 days. This difference in flock age was due to the different market requirements of the participating broiler companies. From each house, a convenience sample of 30 birds was selected by catching 30 available birds from the cool-cell end half of the house. The birds were humanely killed by cervical dislocation. Each carcass was placed into a sterile bio-hazard bag with 250 ml of sterile BPW. The carcass

was vigorously shaken in the bag for 1 min and the whole carcass rinse (GO/WCR) was aseptically transferred into a sterile plastic bottle. The bottles were transported to the laboratory and incubated at 42°C overnight. After the carcass rinse was collected, both of the caeca (GO/CA) and the crop (GO/CP) were aseptically removed from each carcass. Each caecum was placed into a sterile Whirl-Pak® Bag and the crop into a sterile Whirl-Pak® Filter Bag. Caecal and crop samples were processed in the field in a mobile laboratory immediately after sample collection. One caecum was retained for another study; the second caecum was weighed and nine times the weight of tetrathionate (TET) broth (Remel Inc., Lenexa, KS, USA) was added to the sample, stomached for 60 s and incubated at 42°C overnight. Buffered peptone water was added at nine times the weight of the crop to each crop sample, stomached for 60 s and incubated at 42°C overnight.

Post-harvest litter samples and drag swabs

Litter samples (PH/LR) and drag swabs (PH/DS) of the litter were collected in 68 of the sampled houses (at 34 farms) within 4 h after sampling the broilers at the processing plants.

Plant arrival whole carcass rinse, caeca and crop samples

From the 70 flocks sampled at the end of the grow-out stage, four (from two farms) were lost from the study due to company scheduling conflicts. The remaining 66 flocks from 33 farms were sampled upon arrival at the processing plants. The individual flocks numbered from 15 200 to 27 200 birds at this point. The broiler flocks were 48–61 days old, with an average of 56 days. A convenience sample of two birds from each of five cages from each of three livehaul trailers used to transport the flock to the processing plant was selected, totalling 30 broilers from each flock. The birds were humanely killed by cervical dislocation. Carcass rinse samples (PA/WCR), caecal (PA/CA) and crop (PA/CP) samples were collected and processed as previously described.

Pre-chill and post-chill carcass rinse samples

The 66 flocks sampled upon arrival at the processing plants were followed through processing and sampled at two points during processing by whole carcass rinse. The first location was prior to entering the immersion chill tank (PR/CR), at a site between the inside–outside wash cabinet and the chill tank and the second location was as the carcasses exited the chill tank (PO/CR). Samples from two flocks were lost due to a laboratory accident therefore leaving post-chill carcass rinse samples from 64 flocks. At both sampling points in the plants, the first carcass from the flock was sampled at the beginning of the flock pass-

ing through the corresponding processing point. The other 29 carcasses were sampled at a repeating time interval, adjusted for the speed of the processing line. Thus, at both sampling points, collection of the 30 carcass rinse samples was evenly spread across the processing time of the flock. The carcass rinses were collected by removing a carcass from the processing line with newly gloved hands and placing it into a sterile plastic bag with 100 ml of Butterfield's solution. The carcass was vigorously shaken in the bag for 1 min and the rinsate was aseptically transferred into a sterile plastic bottle. Concentrated BPW (10X) was added to the bottle to bring the final concentration to single-strength BPW. Ten millilitres of the sample were removed for other purposes. The bottles were transported to the laboratory and incubated at 42°C overnight.

Salmonella isolation and identification

Salmonella isolation from all samples was performed similarly as described by Rybolt et al. (2005). In short, after overnight incubation, 1 ml from each sample was transferred to 9 ml of TET, vortexed and incubated at 42°C for 48 h. After incubation, 0.1 ml of the TET was transferred to 9.9 ml of Rappaport-Vassiliadis (RV) broth (DIFCO Laboratories, Detroit, MI) and incubated at 42°C overnight. After incubation, one loopful of the RV was plated onto a xylose–lysine tergitol 4 (XLT4) agar plate (Remel Inc., Lenexa, KS), incubated at 37°C overnight and the plates were examined for *Salmonella*-like colonies. A single colony was picked from a positive XLT4 plate and *Salmonella* identity was confirmed biochemically on triple sugar iron and lysine iron agar slants. *Salmonella* isolation was further confirmed by a slide agglutination assay using *Salmonella* O Antiserum Poly A-I & Vi (DIFCO Laboratories, Detroit, MI) as described by the manufacturer.

Sample size calculations

Number of flocks

A broiler flock was the basic unit of analysis and the study was designed to model the associations between *Salmonella* status of the flock and grow-out environment at different points in production and processing with multilevel multiple logistic regression. To allow several fixed-effect predictors to be tested in the final model for each investigated measurement of *Salmonella*, a total of 76 flocks were included in the study. Using a rule of thumb of 10 subjects, or in this case flocks, per explanatory variable (Petrie and Watson, 1999) this number of flocks would allow up to seven explanatory variables to be included in each final model.

Number of samples per flock

The Food Safety and Inspection Service (FSIS) reported that the US national prevalence of *Salmonella* in post-chill broilers from 1998 to 2000 was 10.2% (Progress Report on *Salmonella* Testing of Raw Meat and Poultry Products, 1998–2000, <http://www.fsis.usda.gov/ophs/haccp/salmdata2.htm>). Accordingly, a sample size of 30 birds per flock was adopted in this study for all samples taken from broilers to ensure the detection of *Salmonella* presence, at least one expected positive sample, in each flock with a within-flock prevalence $\geq 9.5\%$ (Cannon and Roe, 1982) and therefore ensured detection of *Salmonella* in all the flocks with *Salmonella* prevalence greater than the national average. The number of samples to be collected from a broiler house (pooled litter samples and drag swabs) was chosen based on experience and practicality.

Statistical procedures

Logistic regression (events/trials syntax) was used to model the relationships between the likelihood of *Salmonella* in samples of each type collected from a bird or house at each sampling point and *Salmonella* frequencies in all the sample types from the flock and its grow-out environment obtained at preceding production stages. Each sample type-point combination that could serve as an outcome and those sample type-point combinations that preceded it and could therefore serve as explanatory variables can be reviewed in Table 1. When a measurement of the flock *Salmonella* status (samples from birds) was analysed as an explanatory variable, a 10% increase in the proportion of positives out of the 30 samples per flock was used as an increment of change. The increment of 10% was preferred to 1% as a more practical and interpretable gradient. For a house environmental sample as an explanatory variable, an increment of change was one *Salmonella* positive sample increase out of the four litter samples or swabs from the house at the sampling point. For example, the likelihood of *Salmonella* in grow-out whole carcass rinses was investigated for associations with each 10% increase in the proportions of positive day-1 tray pads or day-1 chick gastrointestinal tracts from the flock and with each one positive increase out of four day-1 litter samples or day-1 drag swabs from the house.

For each outcome investigated, generalized linear mixed models incorporating hierarchically structured random effects of the farms–complexes–companies, and one or more fixed effect risk factor (designated as the basic model) were fit using the GLIMMIX procedure in SAS[®] 9.1 (SAS Institute Inc., Cary, NC). The random effect factors were incorporated to account for the vari-

ability among the participating farms, complexes and companies, which was not addressed by the fixed effect factors and possible intralevel commonality of these unobserved risk factors at each of the industry's hierarchical levels (Condon et al., 2004). Introduction of the random effects also broadened the sphere of inference of the analysis conducted. Otherwise, model building was performed in general following Hosmer and Lemeshow's (1989) outline. In the screening step for a given outcome, each of the bird or house *Salmonella* status measurements performed at a preceding production point was evaluated in the basic model as a single fixed effect factor. To develop a multiple risk factor model, the risk factors associated with the outcome in the screening step ($P \leq 0.1500$) were considered in the basic model all at one time as the fixed effect factors. After each model fit, the fixed effect variable with the highest *P*-value was removed until a model was developed with all the fixed effect factors significant at $P \leq 0.0500$. Further refinement of the full final model generated for an outcome by the two-step variable selection process was pursued to obtain the most parsimonious final model while preserving its explanatory ability. However, the basic model structure (with three hierarchical random effect factors) was always preserved and only reduction in the fixed effect risk factors was considered. A limited number of tools are available to evaluate the performance of generalized linear mixed models with different numbers of predictors. In this study, the full and reduced candidate models for an outcome were compared using (i) Generalized chi-Square/d.f. (as an approximate measure of the explained residual variation), (ii) Spearman correlation coefficient between the observed and predicted response proportions (considered as an extension of the philosophy of cross-tabulation of the predicted and observed responses for dichotomous outcomes modelled with logistic regression) and (iii) simple squared deviations statistic sum of $[(\text{observed} - \text{expected})^2]$ as suggested by Schukken et al. (2003).

In each final model adopted, significance of each random effect factor was evaluated with a Wald-type test with the test statistic calculated as $[(\text{parameter estimate} / \text{parameter standard error})^2]$ and assumed to follow a chi-square distribution with 1 d.f. under the null hypothesis. The null hypothesis addressed was that the factor made no significant contribution to variability in the outcome given the contribution made by the other variables in the model. Though the primary purpose of forcing the random effect factors into the model was to account for the unobserved risk factors residing at each level of the industry hierarchy, we do provide the results of the Wald-type tests as these may be of interest to the reader.

Results

The results of the screening step of analysis are shown in Table 2. These models measured the strength of association between the likelihood of *Salmonella* in a particular sample type collected later in the production continuum and the frequency of *Salmonella* in a sample type collected earlier in the continuum. For example, the first model shown in Table 2 demonstrated that for each 10% increase in the proportion of *Salmonella* positive day-1 tray pads in the flock, there was a 1.20 ($P < 0.0001$) increased odds of positive grow-out whole carcass rinses. The number of risk factors, i.e. preceding *Salmonella* measurements, with which a particular outcome was associated varied from as few as one in the case of crop samples collected at the end of grow-out to as many as 11 risk factors in the case of pre-chill carcass rinses.

The final models adopted are shown in Table 3. These models retained, as the fixed effect risk factors, only the measurements of *Salmonella* status of the birds and house samples at preceding production stages that were most associated with the outcome. For example, the first model presented in Table 3 demonstrated that the best predictors of *Salmonella* status of whole carcass rinses collected at the end of grow-out were *Salmonella* frequencies in the day-1 tray pads and the day-1 chick gastrointestinal tract samples. For each 10% increase in the proportions of *Salmonella* positive day-1 tray pads and chick gastrointestinal tract samples there was a 1.10 ($P = 0.0167$) and 1.81 ($P < 0.0001$) respectively, increased odds of positive whole carcass rinses to be collected from the flock at the end of grow-out.

Given the fixed effect risk factors in the final models adopted (Table 3), differences among participating grow-out farms, but not the differences on the levels of the complexes or companies, further contributed to the variability in *Salmonella* occurrence in the samples from the broilers at the end of grow-out and upon arrival to the processing plant and in the house environment post-harvest. In particular, differences among farms contributed to the variability in *Salmonella* presence in grow-out whole carcass rinses (Wald-type test $P = 0.0006$), caeca samples ($P = 0.0012$) and crop samples ($P = 0.0340$); as well as in post-harvest litter samples ($P = 0.0546$) and drag swabs of the litter ($P = 0.0265$); and in the plant arrival whole carcass rinses ($P = 0.0001$), caeca samples ($P = 0.0046$) and crop samples ($P = 0.0013$). Such contribution was not observed for the effects on the levels of either participating complexes or companies (all $P > 0.5000$).

As mentioned previously, the associations between the likelihoods of *Salmonella* in samples collected earlier in the production continuum and in the pre-chill and

post-chill carcass rinses were of particular interest. Consequently, the results for these two outcomes will be discussed in more detail.

Pre-chill tank carcass rinses

A sanitation shift was conducted prior to start up of each processing day. On average, four broiler flocks were processed during a shift. In this study, 36% of the flocks sampled prior to the immersion chill tank was the first flocks processed on the line after a sanitation shift, 41% was the second flocks and the remaining 23% was the third to eighth flock on the line following the sanitation shift. It should be acknowledged that the flocks that were not the first on the line after a sanitation shift could have a higher probability of being cross-contaminated with *Salmonella* from the previously processed flocks.

The full final model generated for the pre-chill carcass outcome (after the two-step variable selection process) retained five fixed effect risk factors. This model showed that the likelihood of *Salmonella* on pre-chill broiler carcasses was most associated with higher *Salmonella* contamination of broilers arriving at the plant and of grow-out house environment both prior to the flock's placement and at harvest. Specifically, each 10% increase in the proportion of positive plant arrival whole carcass rinse and crop samples and each additional positive day-1 drag swab, post-harvest litter sample and post-harvest drag swab resulted in a 1.21 ($P = 0.0003$), 1.27 ($P = 0.0022$), 1.40 ($P = 0.0033$), 1.43 ($P < 0.0001$) and 1.23 ($P = 0.0060$) respectively, increased odds of *Salmonella* occurrence in the pre-chill carcass rinses.

Refinement of the full final model for the pre-chill carcass rinse outcome was pursued seeking a more parsimonious model in terms of the fixed effect risk factors, and utilizing the three model performance statistics described above (Table 4). As to relative performance of the three fit statistics, for a pair of candidate models, a change in degree of correlation between the observed and predicted response proportions did not always coincide in direction and relative magnitude with the change in the simple squared deviations statistic. But the latter, as expected, agreed well with changes in the generalized chi-square/d.f.

Examination of the candidate final models provided additional insights into the relative contribution of individual risk factors to the pre-chill carcass rinse outcome and demonstrated that accounting for both the birds' and grow-out environments' *Salmonella* contamination was important to explain the variability in *Salmonella* occurrence in pre-chill carcass rinses. Information provided by the whole carcass rinse sampling upon arrival at the plant was more useful in predicting the pre-chill carcass rinse outcome than that provided by the crop sampling at the

Table 2. Single fixed effect risk factor models of associations between broiler or grow-out environmental sample *Salmonella* status and such measurements at preceding production segments

Outcome ^a	Fixed-effects ^a	n ^b	Units ^c	OR	Wald-type 95% CI (OR)	P-value ^d
GO/WCR	D1/TP	68	10% of 30 sampled	1.20	(1.10, 1.29)	<0.0001
	D1/GI	70	10% of 30 sampled	2.04	(1.61, 2.57)	<0.0001
	D1/DS	70	1 out of 4	1.37	(1.10, 1.71)	0.006
GO/CA	D1/TP	68	10% of 30 sampled	1.22	(1.09, 1.37)	0.0009
	D1/GI	68	10% of 30 sampled	1.35	(1.14, 1.59)	0.0008
GO/CP	D1/TP	68	10% of 30 sampled	1.10	(0.98, 1.24)	0.1141
PH/LR	D1/TP	68	10% of 30 sampled	1.18	(1.02, 1.36)	0.0237
	D1/GI	68	10% of 30 sampled	1.29	(0.94, 1.77)	0.1111
	GO/WCR	68	10% of 30 sampled	1.42	(1.19, 1.69)	0.0002
PH/DS	GO/CA	68	10% of 30 sampled	1.65	(1.24, 2.21)	0.0013
	D1/TP	68	10% of 30 sampled	1.25	(1.10, 1.43)	0.0011
	D1/GI	68	10% of 30 sampled	2.68	(1.67, 4.3)	0.0002
	GO/WCR	68	10% of 30 sampled	1.44	(1.21, 1.72)	0.0002
	GO/CA	68	10% of 30 sampled	Convergence was not reached		
PA/WCR	GO/CP	68	10% of 30 sampled	2.73	(1.18, 6.36)	0.0210
	D1/LR	68	1 out of 4	1.53	(0.99, 2.37)	0.0569
	D1/DS	68	1 out of 4	1.32	(0.96, 1.82)	0.0852
	D1/TP	66	10% of 30 sampled	1.14	(1.06, 1.23)	0.0013
	D1/GI	66	10% of 30 sampled	1.68	(1.38, 2.05)	<0.0001
	GO/WCR	66	10% of 30 sampled	1.47	(1.35, 1.60)	<0.0001
	GO/CA	66	10% of 30 sampled	1.63	(1.39, 1.91)	<0.0001
	GO/CP	66	10% of 30 sampled	1.66	(1.19, 2.32)	0.0042
	PH/LR	66	1 out of 4	1.37	(1.22, 1.53)	<0.0001
	PH/DS	66	1 out of 4	1.54	(1.39, 1.71)	<0.0001
PA/CA	D1/DS	66	1 out of 4	1.33	(1.11, 1.59)	0.0031
	D1/TP	66	10% of 30 sampled	1.14	(1.03, 1.27)	0.0165
	D1/GI	66	10% of 30 sampled	1.28	(1.09, 1.51)	0.0036
	GO/CWR	66	10% of 30 sampled	1.37	(1.24, 1.51)	<0.0001
	GO/CA	66	10% of 30 sampled	1.48	(1.26, 1.74)	<0.0001
	PH/LR	66	1 out of 4	1.58	(1.34, 1.86)	<0.0001
	PH/DS	66	1 out of 4	1.26	(1.10, 1.44)	0.0019
	D1/LR	66	1 out of 4	0.80	(0.60, 1.07)	0.1319
PA/CP	GO/WCR	66	10% of 30 sampled	1.20	(1.10, 1.32)	0.0003
	GO/CA	66	10% of 30 sampled	1.27	(1.07, 1.51)	0.0072
	GO/CP	66	10% of 30 sampled	1.73	(1.24, 2.42)	0.0022
	PH/LR	66	1 out of 4	1.29	(1.10, 1.50)	0.0023
	PH/DS	66	1 out of 4	1.17	(1.03, 1.34)	0.019
	D1/LR	66	1 out of 4	0.81	(0.61, 1.07)	0.1329
	D1/TP	66	10% of 30 sampled	1.18	(1.08, 1.29)	0.0004
PR/CR	D1/GI	66	10% of 30 sampled	2.97	(2.26, 3.91)	<0.0001
	GO/WCR	66	10% of 30 sampled	1.42	(1.30, 1.56)	<0.0001
	GO/CA	66	10% of 30 sampled	1.90	(1.56, 2.32)	<0.0001
	GO/CP	66	10% of 30 sampled	1.77	(1.16, 2.70)	0.0096
	PA/WCR	66	10% of 30 sampled	1.46	(1.35, 1.58)	<0.0001
	PA/CA	66	10% of 30 sampled	1.84	(1.58, 2.14)	<0.0001
	PA/CP	66	10% of 30 sampled	1.70	(1.45, 1.99)	<0.0001
	PH/LR	66	1 out of 4	1.85	(1.62, 2.11)	<0.0001
	PH/DS	66	1 out of 4	1.64	(1.46, 1.84)	<0.0001
	D1/DS	66	1 out of 4	1.19	(0.95, 1.49)	0.1291
PO/CR	GO/CP	64	10% of 30 sampled	0.74	(0.50, 1.08)	0.1151
	PA/CA	64	10% of 30 sampled	1.15	(0.99, 1.34)	0.0655
	PA/CP	64	10% of 30 sampled	1.18	(1.05, 1.33)	0.0082
	PR/CR	64	10% of 30 sampled	1.24	(1.14, 1.34)	<0.0001

Table 2. Continued

Outcome ^a	Fixed-effects ^a	<i>n</i> ^b	Units ^c	OR	Wald-type 95% CI (OR)	<i>P</i> -value ^d
	PH/LR	64	1 out of 4	1.42	(1.24, 1.62)	<0.0001
	D1/LR	64	1 out of 4	1.64	(1.24, 2.15)	0.0009
	D1/DS	64	1 out of 4	0.75	(0.59, 0.95)	0.0198

The models accounted for variability among the production companies, complexes and grow-out farms.

^aD1, day-1; GO, grow-out; PH, post-harvest; PA, plant arrival; PR, pre-chill; PO, post-chill; TP, tray pad; GI, chick gastrointestinal tract; DS, drag swab; LR, litter; CA, Caeca; CP, crop; WCR, whole carcass rinse; CR, carcass rinse.

^bNumber of flocks for which the outcome was modelled.

^cModelled increment of increase in *Salmonella* positive samples in predictors.

^dOnly fixed-effect factors associated with the outcome ($P \leq 0.1500$) are listed.

Table 3. Final models of associations between broiler flocks or grow-out environmental *Salmonella* status and such measurements at preceding production segments

Outcome ^a	Fixed effect ^a	<i>n</i> ^b	Units ^c	OR	Wald-type 95% CI (OR)	<i>P</i> -value	Variability among farms <i>P</i> -value ^d
GO/WCR	D1/TP	68	10% of 30 sampled	1.10	(1.02, 1.2)	0.0167	0.0006
	D1/GI	68	10% of 30 sampled	1.81	(1.42, 2.3)	<0.0001	
GO/CA	D1/TP	68	10% of 30 sampled	1.17	(1.04, 1.31)	0.01	0.0012
	D1/GI	68	10% of 30 sampled	1.26	(1.06, 1.49)	0.0095	
GO/CP	D1/TP	68	10% of 30 sampled	1.10	(0.98, 1.24)	0.1141	0.034
PH/LR	GO/WCR	68	10% of 30 sampled	1.42	(1.19, 1.69)	0.0002	0.0546
PH/DS	D1/GI	68	10% of 30 sampled	2.11	(1.31, 3.38)	0.0031	0.0265
	GO/WCR	68	10% of 30 sampled	1.31	(1.07, 1.59)	0.0104	
PA/WCR	GO/WCR	66	10% of 30 sampled	1.36	(1.24, 1.48)	<0.0001	0.0001
	PH/DS	66	1 out of 4	1.35	(1.20, 1.51)	<0.0001	
PA/CA	GO/WCR	66	10% of 30 sampled	1.29	(1.17, 1.44)	<0.0001	0.0046
	PH/LR	66	1 out of 4	1.38	(1.16, 1.64)	<0.0006	
PA/CP	GO/CP	66	10% of 30 sampled	1.84	(1.31, 2.56)	0.0008	0.0013
	PH/LR	66	1 out of 4	1.32	(1.13, 1.55)	0.0009	
PR/CR	PA/WCR	66	10% of 30 sampled	1.21	(1.1, 1.33)	0.0003	0.0037
	PA/CP	66	10% of 30 sampled	1.27	(1.1, 1.47)	0.0022	
	PH/LR	66	1 out of 4	1.43	(1.23, 1.66)	<0.0001	
	PH/DS	66	1 out of 4	1.23	(1.07, 1.41)	0.006	
	D1/DS	66	1 out of 4	1.40	(1.13, 1.74)	0.0033	
PO/CR	PH/LR	64	1 out of 4	1.55	(1.34, 1.79)	<0.0001	0.0012
	D1/LR	64	1 out of 4	2.04	(1.53, 2.71)	<0.0001	

The models accounted for variability among the production companies, complexes and grow-out farms.

^aD1, day-1; GO, grow-out; PH, post-harvest; PA, plant arrival; PR, pre-chill; PO, post-chill; TP, tray pad; GI, chick gastrointestinal tract; DS, drag swab; LR, litter; CA, Caeca; CP, crop; WCR, whole carcass rinse; CR, carcass rinse.

^bNumber of flocks for which the outcome was modelled.

^cModelled increment of increase in *Salmonella* positive samples in predictors.

^dSignificance of variability among the companies and complexes is discussed in the text.

same point. From the reduced candidate models, a model with three fixed effect factors (plant arrival whole carcass rinses and post-harvest litter samples and drag swabs) performed the best and closest to the full model, which additionally included plant arrival crop samples and day-1 drag swabs. The residuals (obtained while accounting for the random effects factors) of these two models were

examined and the relationships between the predicted and observed responses were further investigated graphically. For the full model, a plot of the nonparametric kernel density estimate of the Pearson residuals' distribution demonstrated that the residuals' distribution was zero-centred and well-bell-shaped with only slight skewing to the right due to a few (7.58%) relatively large ($>|2.0|$)

Table 4. Relative performance of candidate models of the risk factors associated with likelihood of *Salmonella* in broiler carcass rinses prior to the immersion chill tank ($n = 66$)

Fixed effect factors ^a	Generalized χ^2 /d.f.	Σ (observed–predicted) ²	Correlation observed & predicted ^b
PA/WCR + PA/CP + PH/LR + PH/DS + D1/DS ^c	1.47	0.264	0.969
PA/WCR + PA/CP	2.05	0.526	0.952
PH/LR + PH/DS + D1/DS	1.74	0.387	0.971
PA/WCR + PA/CP + PH/LR	1.62	0.318	0.966
PA/WCR + PA/CP + PH/LR + PH/DS	1.54	0.285	0.969
PA/WCR + PA/CP + PH/LR + D1/DS	1.6	0.316	0.9675
PA/CP + PH/LR + PH/DS	1.74	0.396	0.969
PA/WCR + PH/LR + PH/DS	1.55	0.284	0.971
PA/WCR + PH/LR + D1/DS	1.62	0.321	0.971

The models accounted for variability among the production companies, complexes and grow-out farms.

^aD1, day-1; GO, grow-out; PH, post-harvest; PA, plant arrival; PR, pre-chill; PO, post-chill; TP, tray pad; GI, chick gastrointestinal tract; DS, drag swab; LR, litter; CA, Caeca; CP, crop; WCR, whole carcass rinse; CR, carcass rinse.

^bAll correlation coefficients P -value <0.001.

^cFinal model.

positive residuals. A plot of the predicted versus observed responses for the full model demonstrated a good agreement between the two. Distribution of residuals of this reduced model demonstrated a lower compliance with normality than that of the full model. In particular, the plot of non-parametric kernel density estimate of distribution of the reduced model's Pearson residuals demonstrated that the centre of the distribution was moved to the left from zero, although there were still several relatively large positive residuals. Therefore, the full model (five fixed effect factors) was adopted as the final model for pre-chill carcass rinses, acknowledging that the majority of variability in this outcome was explained by three predictors: plant arrival whole carcass rinses, post-harvest litter samples and post-harvest drag swabs.

Differences among the grow-out farms ($P = 0.0037$), but not at the levels of the production complexes or companies (both P -values >0.9000), contributed to the variability in *Salmonella* status of the carcasses of birds when they reached the pre-chill tank point during processing.

Post-chill tank carcass rinses

The full final model developed for the post-chill carcass rinse outcome included four fixed effect factors: plant arrival crop, post-harvest litter sample, grow-out crop and day-1 litter sample. An increase in *Salmonella* posi-

Table 5. Relative performance of candidate models of the risk factors associated with likelihood of *Salmonella* in broiler carcass rinses upon exit from the immersion chill tank ($n = 64$)

Fixed effect factors ^a	Generalized χ^2 /d.f.	Σ (observed–predicted) ²	Correlation observed & predicted ^b
PA/CP + GO/CP + PH/LR + D1/LR	2.30	0.501	0.886
PA/CP + PH/LR + D1/LR	2.48	0.542	0.879
PA/CP + PH/LR	2.74	0.856	0.875
PA/CP + D1/LR	2.92	0.914	0.879
PA/CP + GO/CP + PH/LR	2.71	0.849	0.874
PA/CP + GO/CP + D1/LR	2.59	0.783	0.8935
PH/LR + D1/LR ^c	2.42	0.548	0.878

The models accounted for variability among the production companies, complexes and grow-out farms.

^aD1, day-1; GO, grow-out; PH, post-harvest; PA, plant arrival; PR, pre-chill; PO, post-chill; TP, tray pad; GI, chick gastrointestinal tract; DS, drag swab; LR, litter; CA, Caeca; CP, crop; WCR, whole carcass rinse; CR, carcass rinse.

^bAll correlation coefficients P -value <0.0010.

^cFinal model.

tive grow-out crop samples was negatively associated with the likelihood of *Salmonella* positive post-chill carcass rinses. This relationship was opposite to that observed for the pre-chill carcass rinse outcome. However, the significance of grow-out crop samples as the predictor for the post-chill carcass rinse outcome depended upon simultaneous presence of the plant arrival crop sample factor in the model (i.e. if the plant arrival crop variable was dropped from the full model, the grow-out crop variable did not exhibit a significant ($P > 0.0500$) association with the response). Further examination of the candidate models for the post-chill carcass rinse outcome (Table 5) demonstrated that the reduced two-fixed effect factors model containing only post-harvest and day-1 litter samples performed almost the same as the full four-fixed effect factors model.

The residuals (obtained while accounting for the random effect factors) of the full final model and the best reduced (post-harvest and day-1 litter samples only) models for the post-chill carcass rinse outcome were further examined. For the full model, the plot of non-parametric kernel density estimate of the Pearson residuals' distribution showed that the distribution did not greatly deviate from a bell-shape, but was centred slightly to the left from zero. Few relatively large positive residuals were present, but the majority of the residuals were negative. The distribution of the Pearson residuals for the reduced model was closer to a normal distribution than that of the full model. The centre of the Pearson residuals' distribution of the reduced model was closer to zero than that

for the full model, although several relatively large positive residuals were still present. Plots of the observed versus predicted responses for these two models were also examined. It was concluded that the reduced model could substitute for the full four-fixed effect risk factor model without a loss in the predictions and the former was adopted as the final model for the post-chill carcass rinse outcome. Therefore, in this study the likelihood of *Salmonella* contaminated broiler carcasses exiting the immersion chill tank was most associated with the frequencies of *Salmonella* in the litter in the grow-out house on the day of the flock's harvest and prior to its placement. Specifically, each additional positive post-harvest and day-1 litter sample, was associated with a 1.55 ($P < 0.0001$) and 2.04 ($P < 0.0001$), respectively, increased odds of *Salmonella* positive post-chill carcass rinses.

Investigation of the candidate models for the post-chill carcass rinse outcome suggested that, of samples collected from birds, frequency of *Salmonella* positive crop samples at arrival to the plant was the factor most associated with the likelihood of *Salmonella* contaminated post-chill carcass rinses.

The differences among the grow-out farms on which the flocks were raised ($P = 0.0012$), but not the differences at the levels of the production complexes or companies (both P -value > 0.5000), contributed to the variability in *Salmonella* status of the carcasses of birds when they reached the post-chill point in processing, the final point of the production continuum.

Discussion

The objective of this study was to investigate how the likelihoods of *Salmonella* presence in different sample types at different points in the broiler production continuum were related. Of particular interest was determining how the *Salmonella* statuses of broiler carcasses entering and exiting the immersion chill tank were related to the frequencies of *Salmonella* in both bird and environmental samples collected in earlier segments of production. However, ascertaining the associations between *Salmonella* occurrence at these earlier segments and in different sample types, not just in pre- and post-chill carcass rinses, allows us to better understand the ecology of *Salmonella* within the broiler production continuum. These associations can be exploited to identify interventions and the best places to implement them to effectively control *Salmonella* in broiler production.

The results of this study indicate that the likelihoods of *Salmonella* in whole carcass rinse and caecal samples from a broiler flock at the end of grow-out were associated with *Salmonella* status of the flock at the time of delivery to the farm, as measured by chick tray pad and gastroin-

testinal tract of day-old bird samples. However, the likelihood of *Salmonella* being present in these grow-out samples was not associated with the frequency of *Salmonella* in the house environment prior to placement, as measured by the litter samples and drag swabs. The likelihood of *Salmonella* in crop samples in grow-out was only associated with the degree of contamination of the tray pads. These associations were established after accounting for the variability among participating companies, complexes and grow-out farms.

Temporally, the post-harvest litter samples and drag swabs were taken about 1 week after the collection of the grow-out whole carcass rinse, caecum and crop samples. Accordingly, the post-harvest litter samples and drag swabs were modelled as outcomes of samples collected at the end of grow-out and on the day of placement. The likelihood of *Salmonella* in post-harvest litter samples was associated with grow-out whole carcass rinses while the likelihood of positive post-harvest drag swabs was associated with grow-out whole carcass rinses and day-1 gastrointestinal tracts. Although it is reasonable that the *Salmonella* status of chicks on the day of placement could impact the status of drag swabs collected post-harvest, it would seem the direction of the association is incorrect for whole carcass rinses. Nonetheless, the identification of the associations was thought to be useful even if the implied cause and effect relationship is not likely to exist. Post-harvest litter samples and drag swabs were considered as explanatory variables in models for plant arrival, pre-chill and post-chill sample outcomes as they were considered measures of the status of the house environment at the time of harvest, which preceded the processing plant sample collection, even if they were actually collected after plant samples.

The likelihoods of *Salmonella* in whole carcass rinse, crop and caecal samples collected from the broiler flocks as they arrived at the processing plant were associated with the *Salmonella* status of samples from birds at the end of grow-out and contamination of the grow-out house environment on the day of harvest. However, the likelihoods of the pathogen being present in the birds as they arrived at the processing plant were not associated with measurements taken on the day the flock was placed on the farm. Particularly, the likelihood of *Salmonella* in the whole carcass rinses collected at plant arrival was associated with *Salmonella* frequencies in whole carcass rinses at the end of grow-out and in drag swabs taken on the day of processing. It is reasonable that *Salmonella* contamination of the exterior of the birds at plant arrival would be associated with their exterior contamination in grow-out and with contamination of the grow-out environment, especially when one considers the drag swab to be a measure of *Salmonella* contamination of the surface

of the litter to which the exteriors of the birds had been directly exposed.

Analysis of the caecal and crop samples collected upon arrival at the plant suggested that *Salmonella* contaminating the litter in the grow-out house near the time of harvest may be important in pre-disposing *Salmonella* positive crops and caeca in the broiler flock arriving for processing. This may be a consequence of the litter pecking behaviour during the feed withdrawal period that is usually implemented several hours prior to the harvest to decrease intestinal contents during processing as observed by Corrier, et al. (1999).

Salmonella contamination of pre-chill broiler carcasses was associated with a variety of factors at the screening step of analysis. However, the final model adopted for this outcome showed that the likelihood of *Salmonella* on pre-chill carcasses was primarily dependent upon the extent of *Salmonella* contamination of the exterior of broilers arriving for processing and the proportion of birds with *Salmonella* positive crops at this time. Furthermore, it was dependent upon *Salmonella* levels in the environment of the house, both at the time of harvest and prior to placement of the flock. It is interesting to note that while relationships of the *Salmonella* status of samples collected at plant arrival only extended back to measures taken at the end of grow-out, the pre-chill flock status was associated with measures from both the day of harvesting as well as the day of placement.

The variety of factors that appeared to be associated with the likelihood of *Salmonella* on pre-chill broiler carcasses may reflect many possibilities for self-contamination of a processed carcass from its exterior and interior surfaces and cross-contamination among the carcasses on the processing line. Sanitation procedures, such as sprayers, are usually equipped along the lines prior to the immersion chill tanks to reduce bacterial load on the processed carcasses and may add to the variability in the flocks' *Salmonella* status due to differences in effectiveness. The effectiveness depends on the design of the devices, antimicrobial compounds used and their concentration in the spray at a given time during processing (Notermans et al., 1980; Lillard, 1989; James et al., 1992a,b; Waldroup, 1993; Salvat et al., 1997; Sarlin et al., 1998). *Salmonella* carried over from flocks processed earlier on the line and even the occurrence of *Salmonella* serotypes not observed in the flock during grow-out nor in a flock processed on the line right before the sampled flock, have been reported (Morris et al., 1969; Dougherty, 1976; Sarlin et al., 1998).

The set of risk factors associated with the likelihood of *Salmonella*-contaminated broiler carcasses exiting the immersion chill tank differed from that for the pre-chill point in this analysis. Interestingly, frequency of *Salmo-*

nella on pre-chill carcasses was not retained as a fixed effect risk factor in the final model for *Salmonella* status of the flock post-chill. Although one might anticipate that the pre-chill rinses would be the best predictor of the post-chill contamination, it appeared that the immersion chilling process could disrupt this relationship. It may be that some of the bacteria on or in the chicken skin were removed during immersion chilling. Re-contamination and cross-contamination among the carcasses could occur as well. Management of the immersion chill tank and chlorination of the chill water are known to affect the extent of cross-contamination among the carcasses in the chill tank (Thiessen et al., 1984; James et al., 1992a, 1992b; Waldroup, 1993).

In this study, the most parsimonious model of the risk factors associated with the likelihood of *Salmonella* in post-chill carcass rinses identified the frequencies of *Salmonella* in grow-out house litter samples post-harvest and on the day the birds arrived at the farm as the most significant predictors. Alternative candidate models demonstrated that the frequency of *Salmonella* positive crops in the birds at plant arrival was an informative predictor as well (Table 5). However, the relationships between *Salmonella* presence in the broiler crops a week before harvest and at plant arrival appeared to be complex. The *Salmonella* status of crops in birds at plant arrival was, in turn, associated with the degree of litter contamination with *Salmonella* on the day of harvest. It appears that investigation into cycling of *Salmonella* between the broiler flock and the litter in the grow-out house, and elaboration of a broiler litter management protocol to control *Salmonella* in the litter, may be a fruitful approach to reduce *Salmonella* contamination of post-chill broiler carcasses.

After accounting for the risk factors identified, the variability in *Salmonella* status of the grow-out broilers and then carcasses from the flock appeared to be enhanced by the differences among the grow-out farms. No further significant contribution was observed due to impacts of the differences at the levels of the complexes or companies. This suggested that the other risk factors affecting *Salmonella* status of the broilers in grow-out and the carcasses in processing are likely to be at the farm level.

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